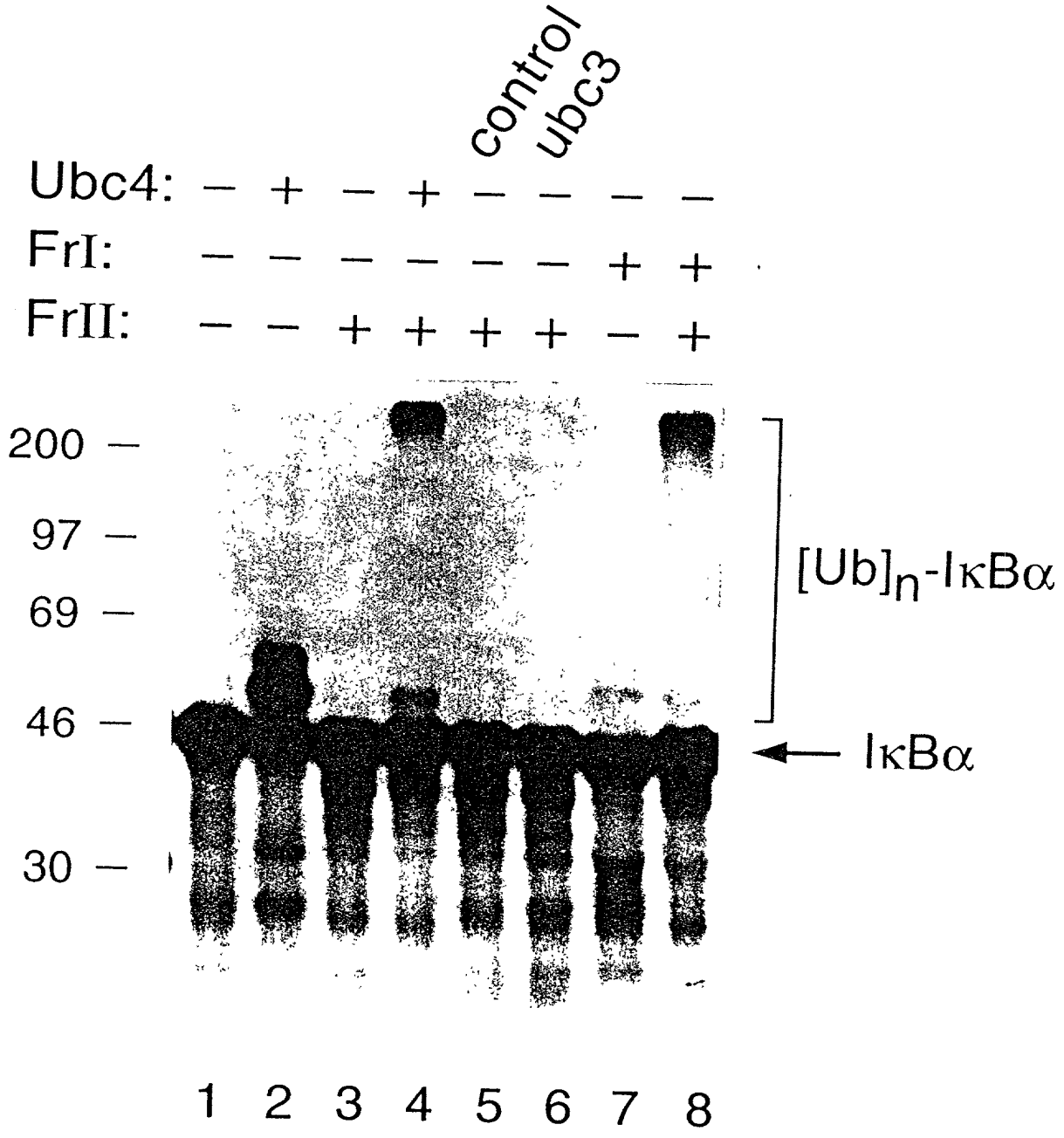

control
ubc3
Ubc4: − + − + − − − −
FrI: − − − − − − + +
FrII: − − + + + + − +
200 −
97 −
69 −
46 −
30 −
[Ub]n-IκBα
IκBα
1 2 3 4 5 6 7 8

FIG. 1

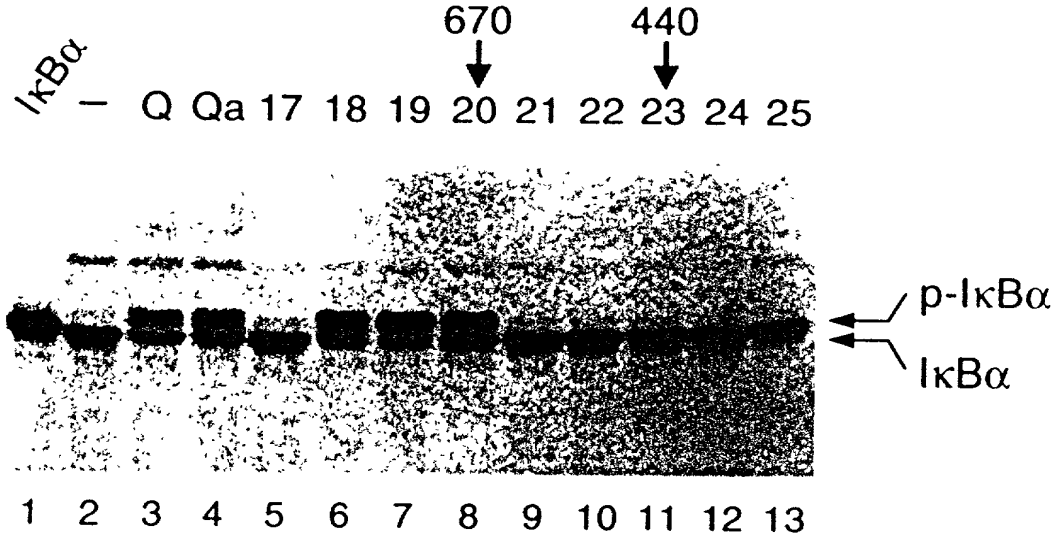

670
440
IκBα
– Q Qa 17 18 19 20 21 22 23 24 25
p-IκBα
IκBα
1 2 3 4 5 6 7 8 9 10 11 12 13

FIG.2A

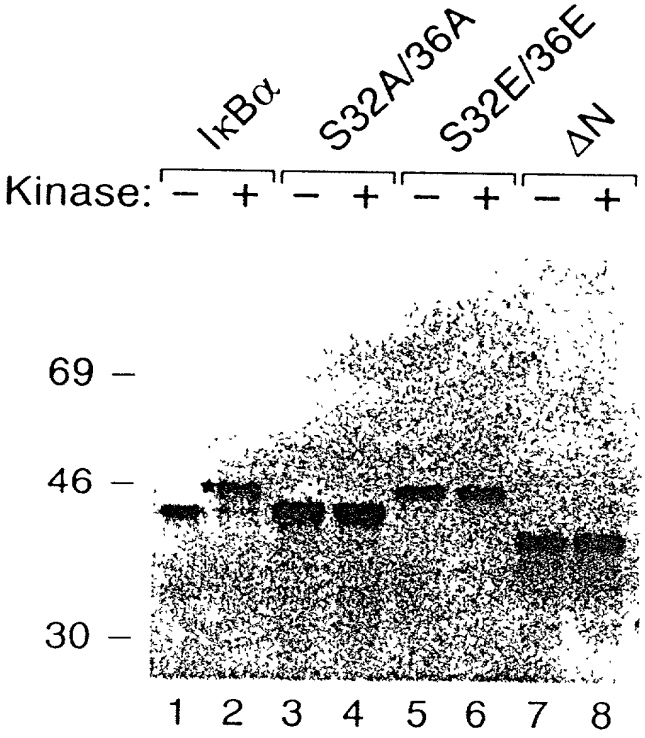

IκBα
S32A/36A
S32E/36E
ΔN
Kinase: – + – + – + – +
69 –
46 –
30 –
1 2 3 4 5 6 7 8

FIG.2C

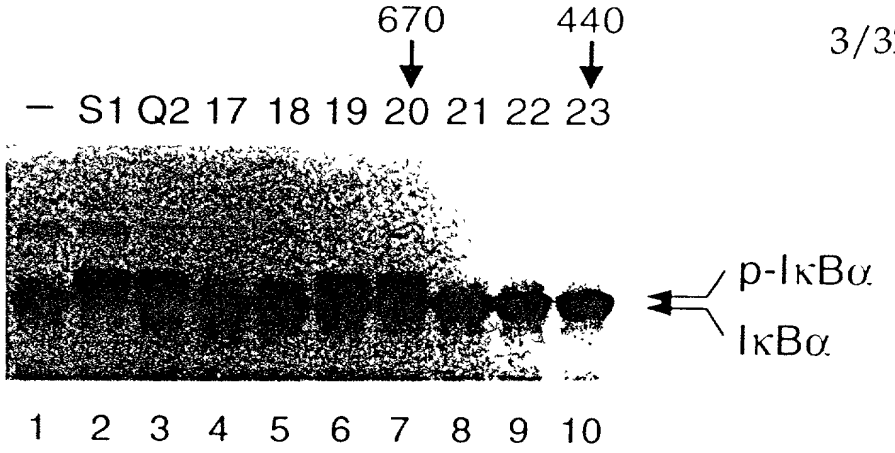


FIG.2B

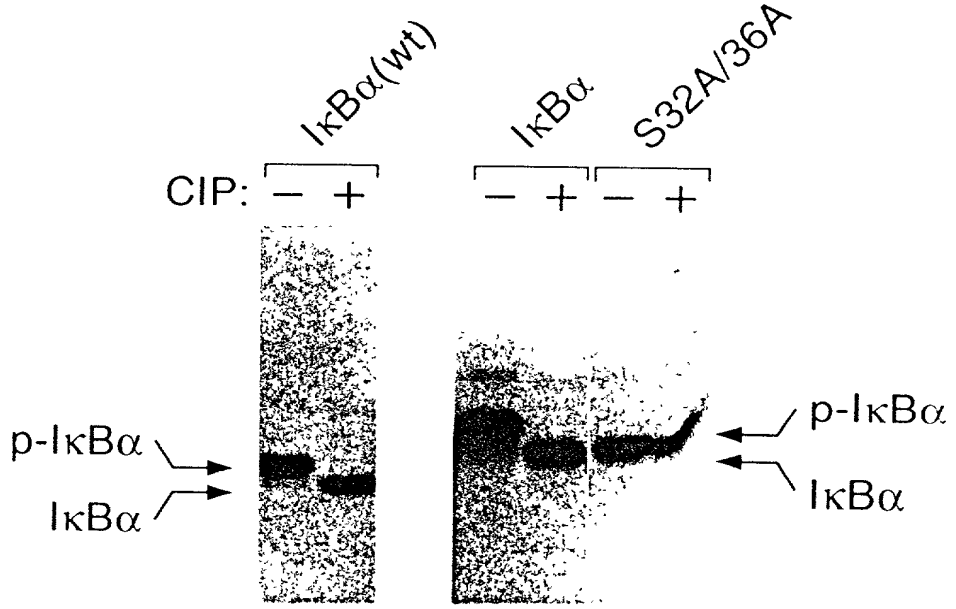


FIG.2D

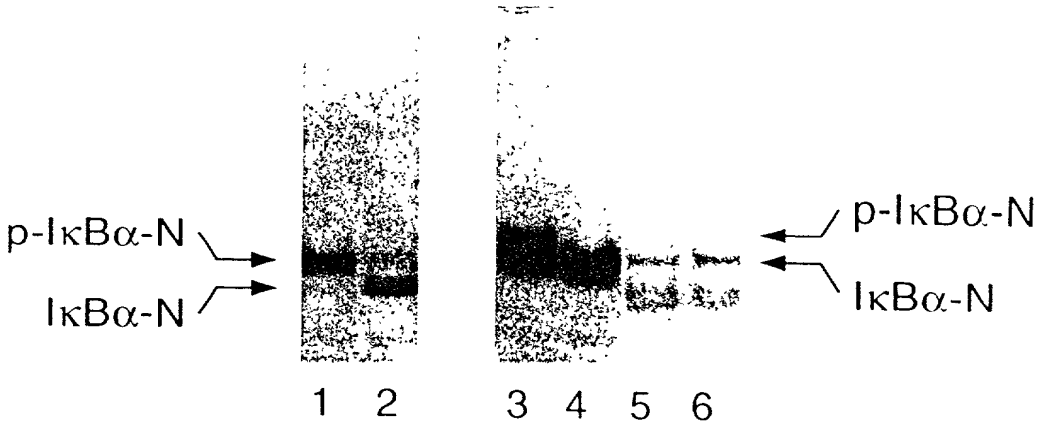


FIG.2E

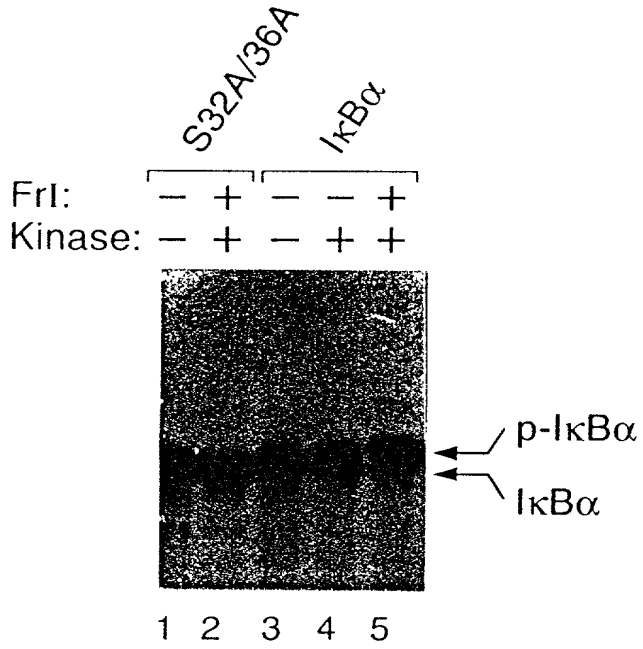

S32A/36A
IκBα
FrI: − + − − +
Kinase: − + − + +
p-IκBα
IκBα
1 2 3 4 5

FIG.3A

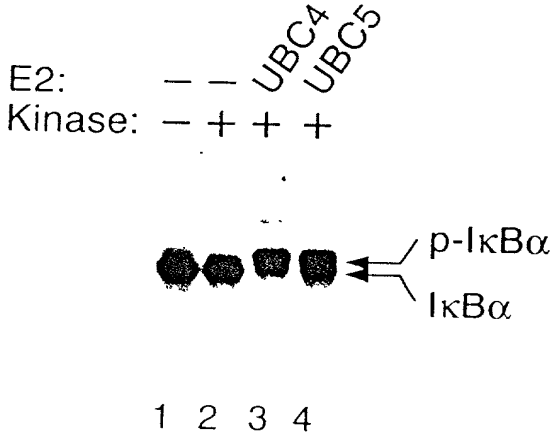

E2: − − UBC4 UBC5
Kinase: − + + +
p-IκBα
IκBα
1 2 3 4

FIG.3B

FIG.3C

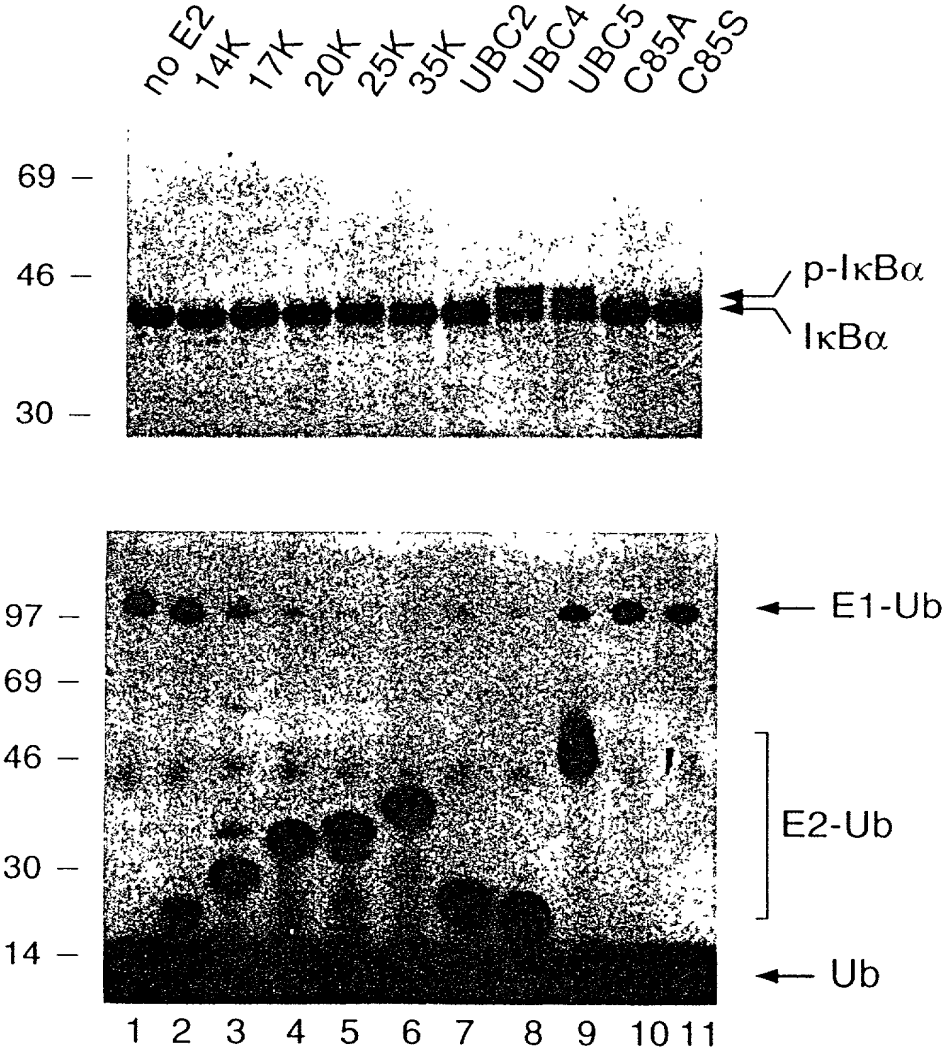

no E2
14K
17K
20K
25K
35K
UBC2
UBC4
UBC5
C85A
C85S
69
46
30
p-IκBα
IκBα
97
69
46
30
14
E1-Ub
E2-Ub
Ub
1 2 3 4 5 6 7 8 9 10 11

FIG.3D

SUBSTITUTE SHEET (RULE 26)

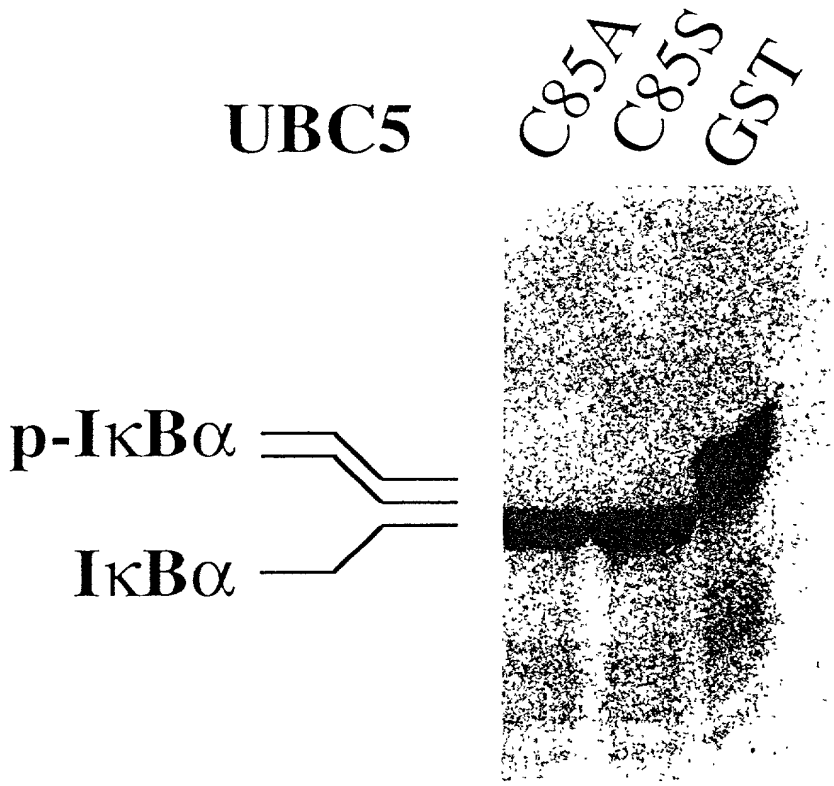


FIG.3E

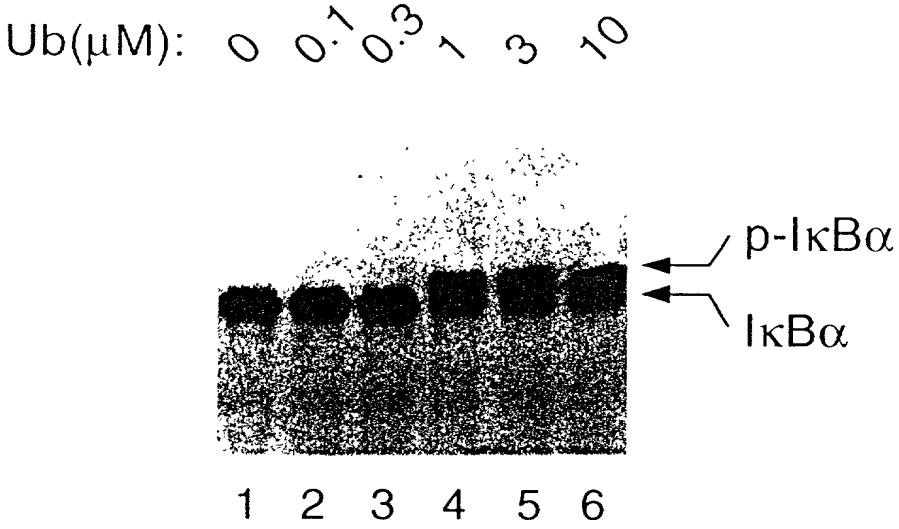

Ub(μM): 0 0.1 0.3 1 3 10
p-IκBα
IκBα
1 2 3 4 5 6

FIG.4A

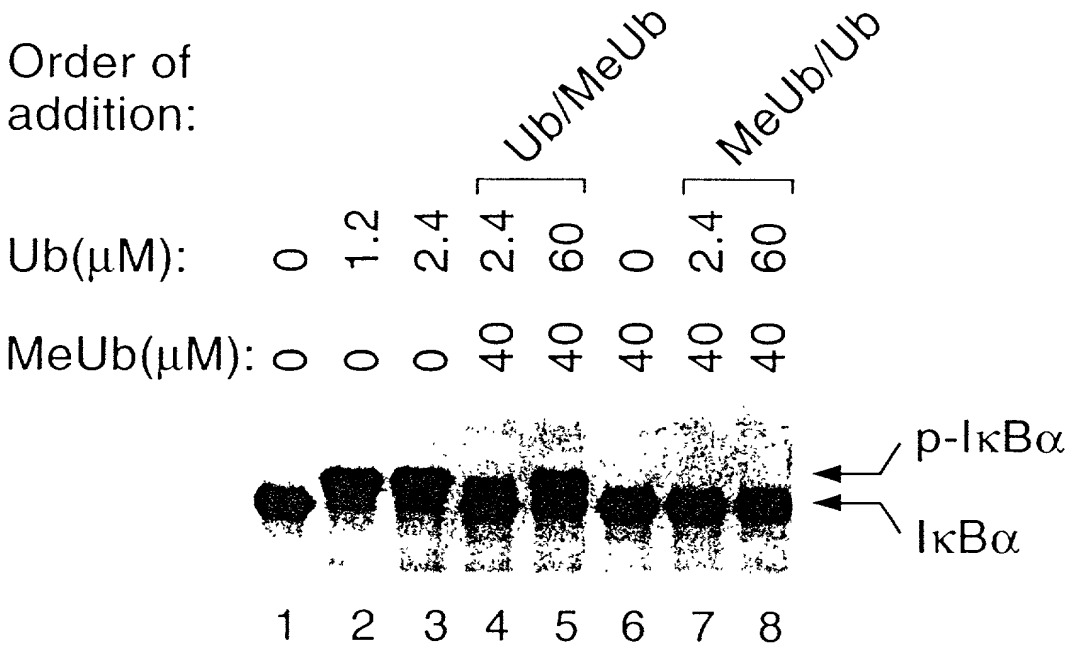

Order of addition:
Ub/MeUb
MeUb/Ub
Ub(μM): 0 1.2 2.4 2.4 60 0 2.4 60
MeUb(μM): 0 0 0 40 40 40 40 40
p-IκBα
IκBα
1 2 3 4 5 6 7 8

FIG.4B

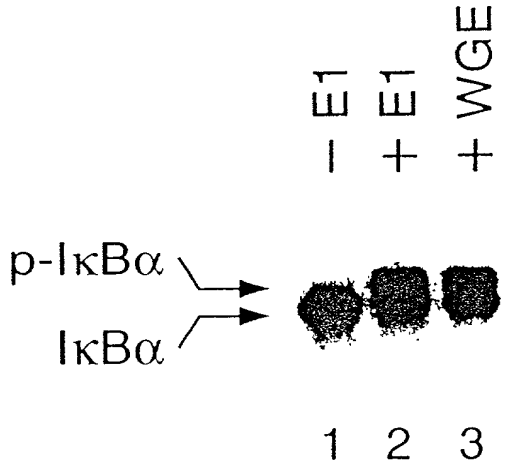


FIG.5A

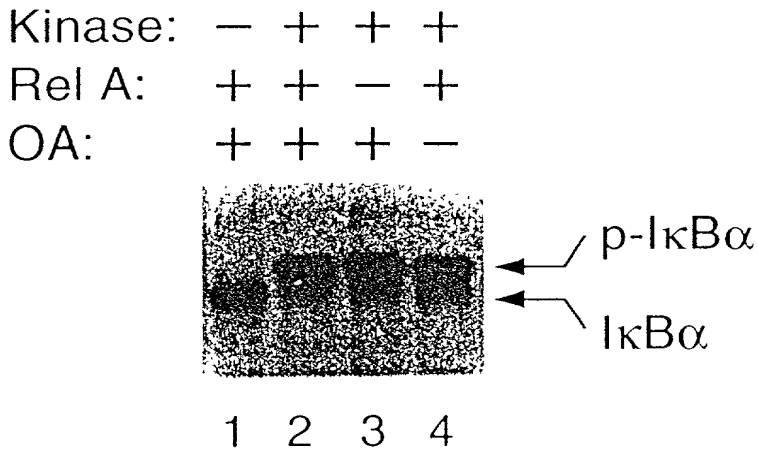


FIG.5B

SUBSTITUTE SHEET (RULE 26)

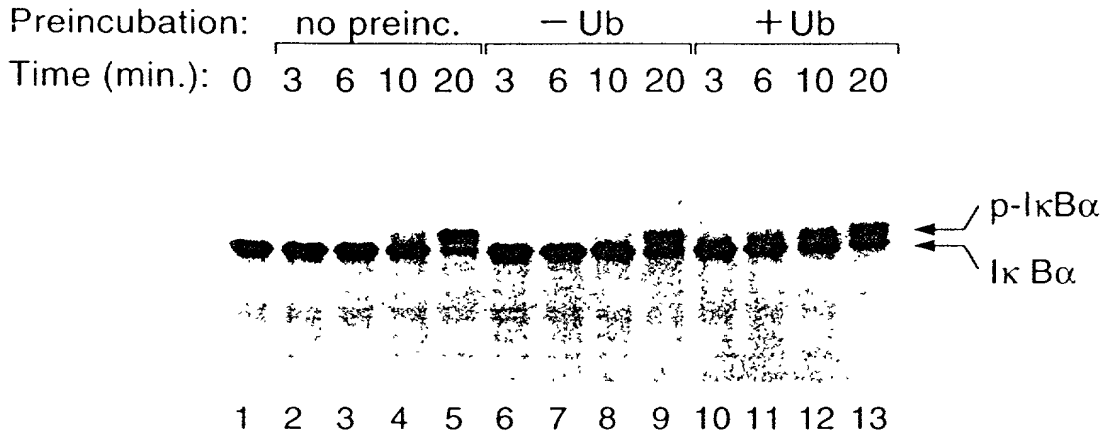


FIG.6A

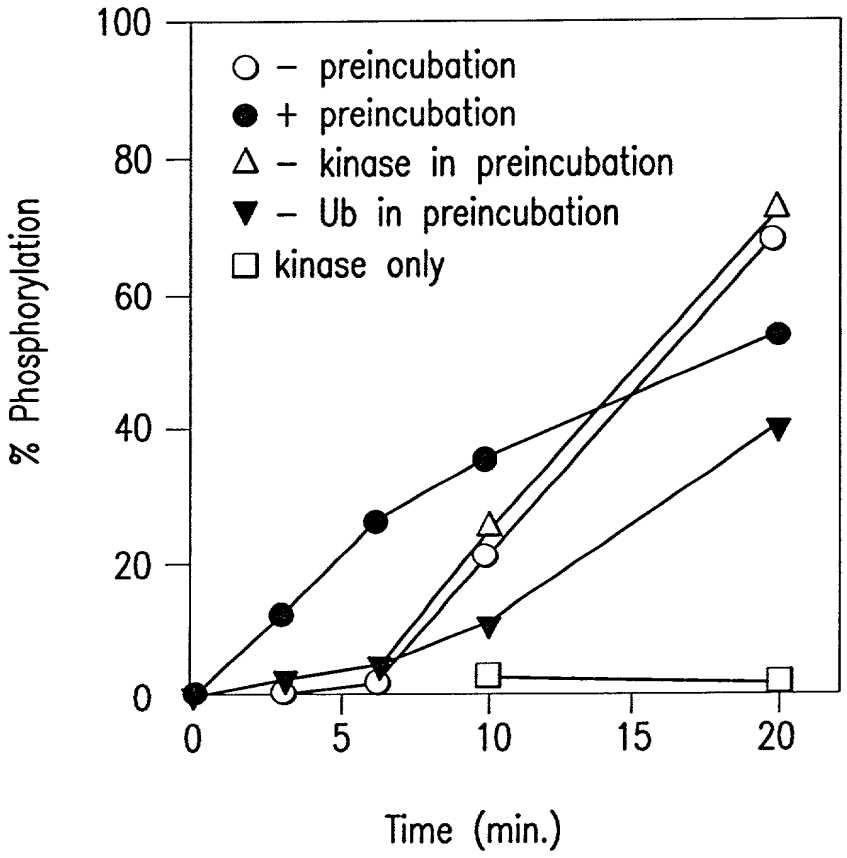

100
80
60
40
20
0
% Phosphorylation
○ – preincubation
● + preincubation
△ – kinase in preincubation
▼ – Ub in preincubation
□ kinase only
0
5
10
15
20
Time (min.)

FIG. 6B

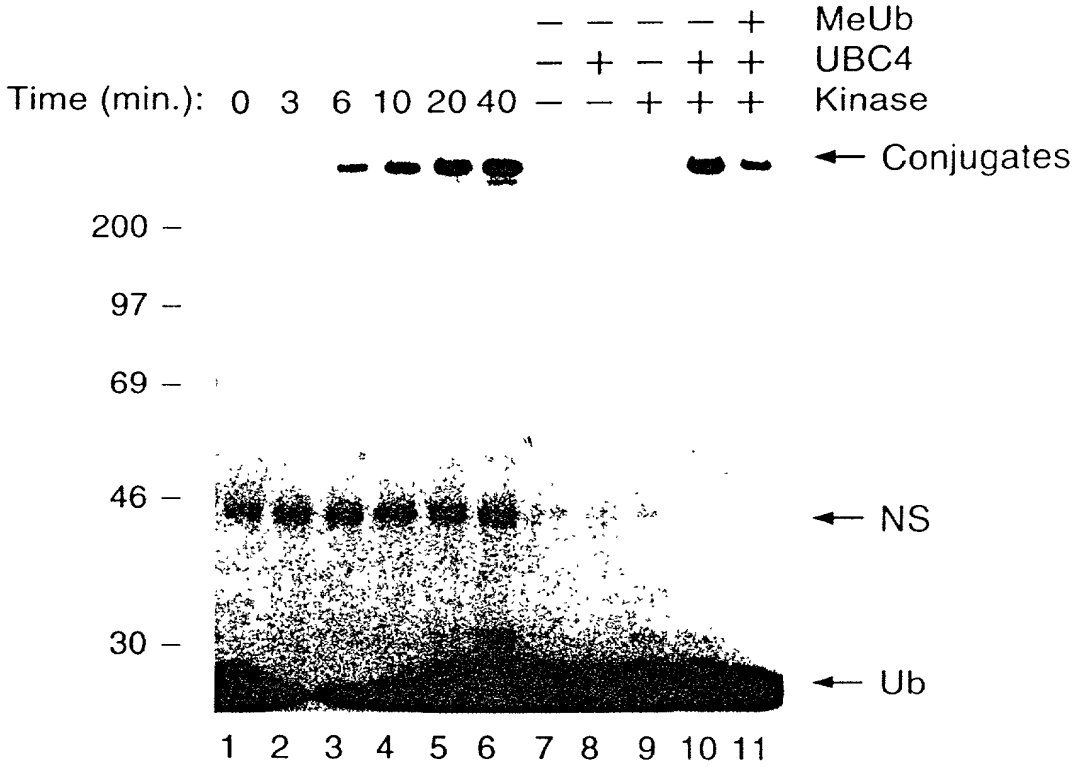

MeUb
UBC4
Kinase
Time (min.): 0 3 6 10 20 40
Conjugates
200
97
69
46
30
NS
Ub
1 2 3 4 5 6 7 8 9 10 11

FIG.7A

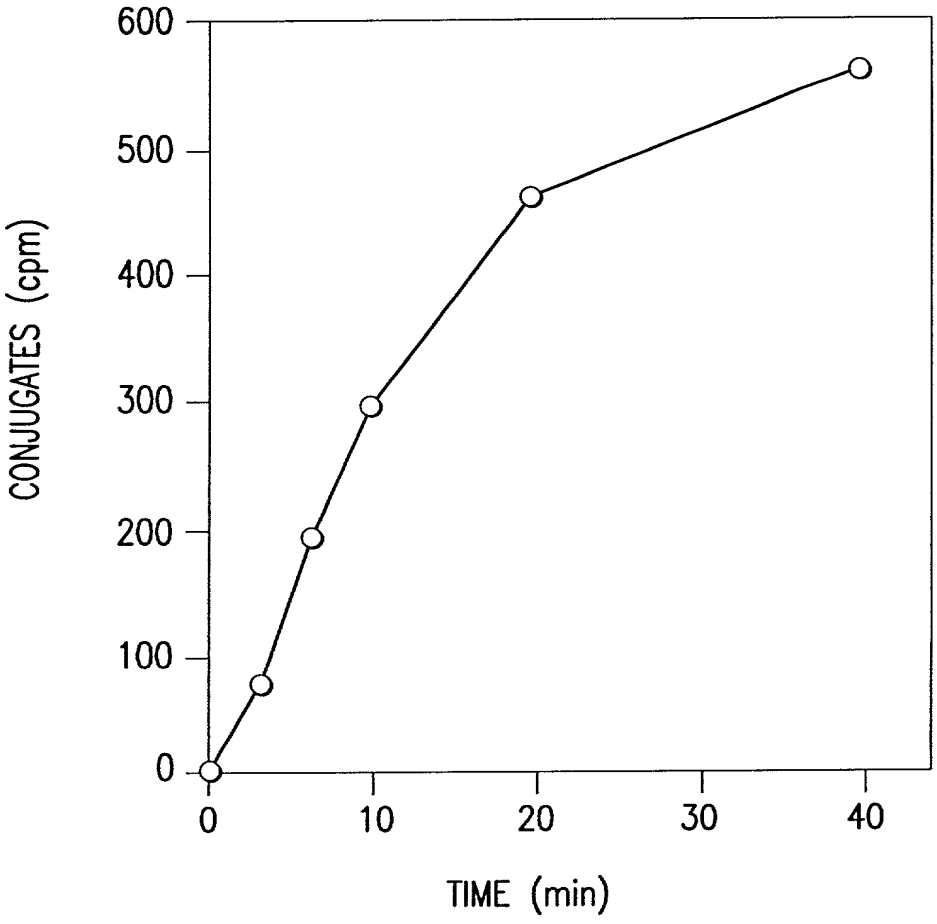


FIG. 7B

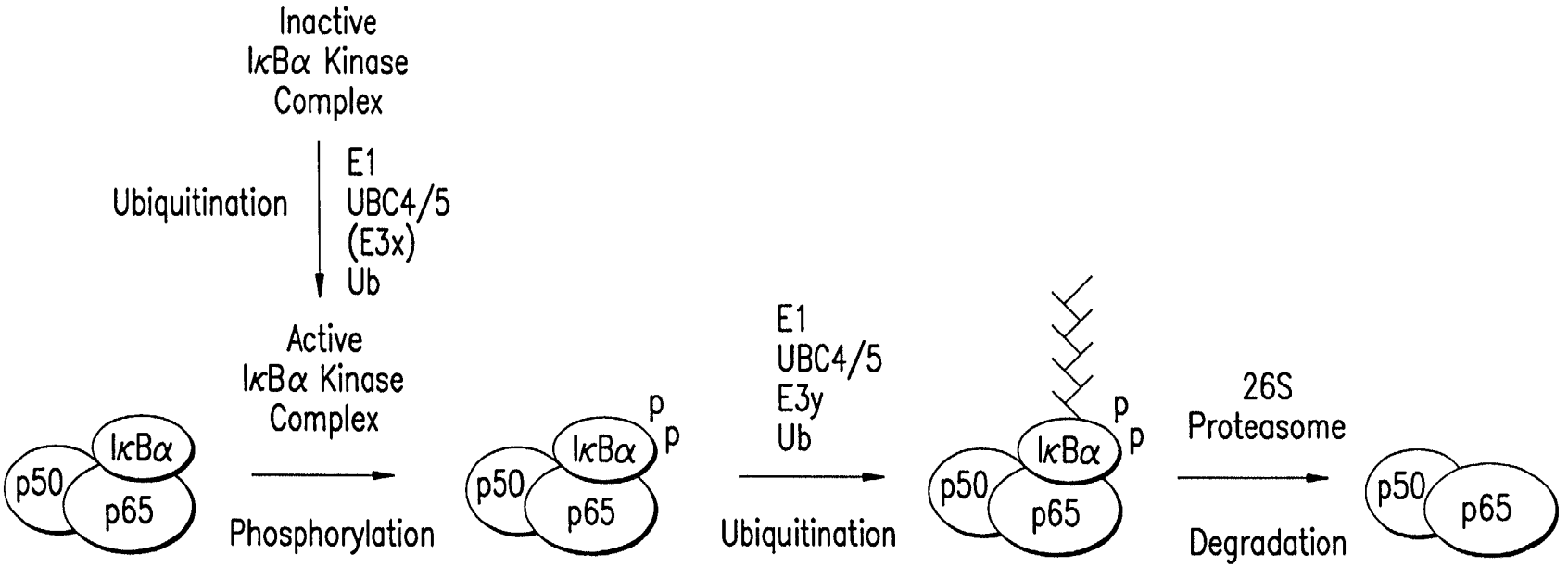

Inactive
IκBα Kinase
Complex
Ubiquitination
E1
UBC4/5
(E3x)
Ub
Active
IκBα Kinase
Complex
IκBα
p50
p65
Phosphorylation
p
p
E1
UBC4/5
E3y
Ub
Ubiquitination
26S
Proteasome
Degradation

FIG. 8

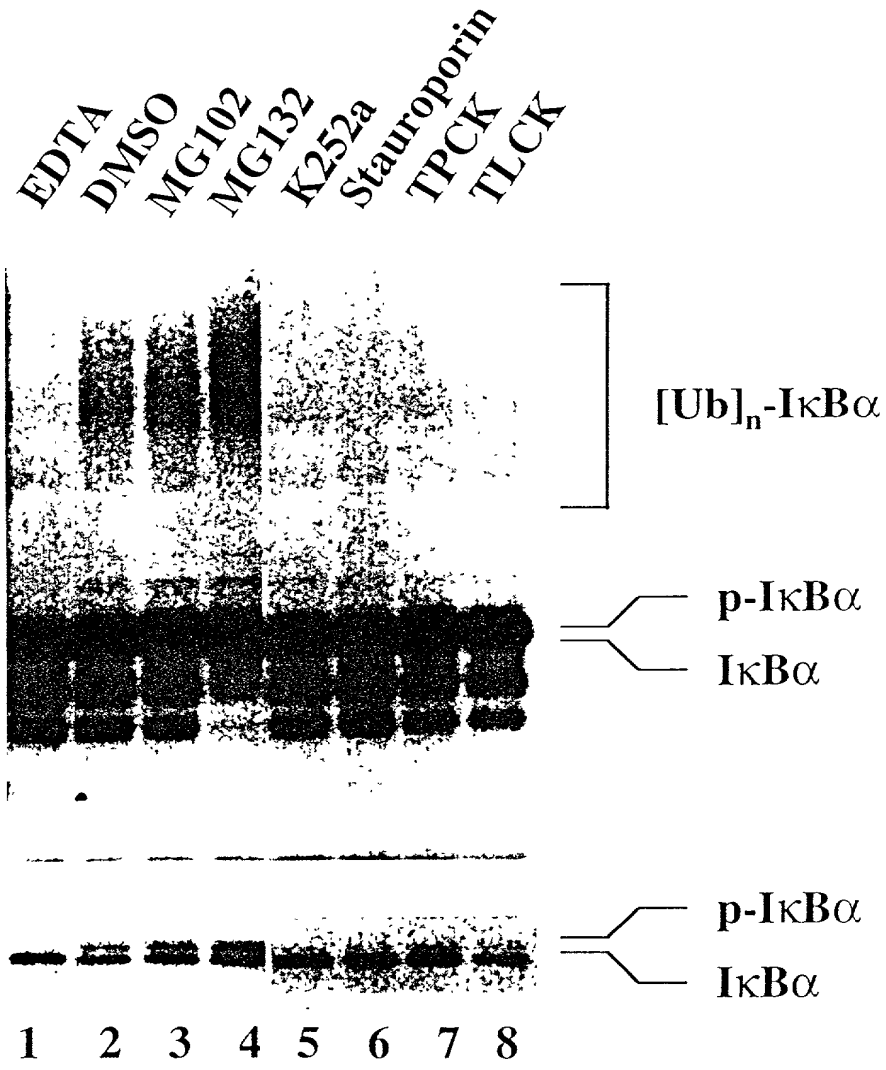
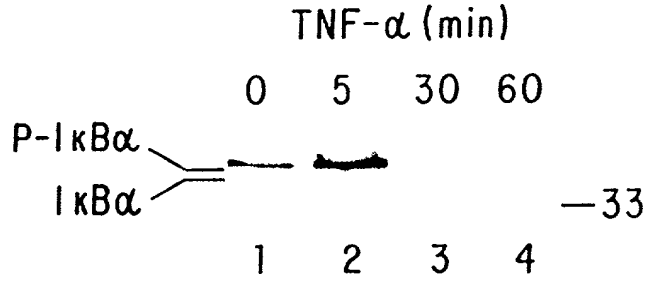

EDTA
DMSO
MG102
MG132
K252a
Stauroporin
TPCK
TLCK
[Ub]n-IκBα
p-IκBα
IκBα
p-IκBα
IκBα
1 2 3 4 5 6 7 8

FIG.9

FIG.10A



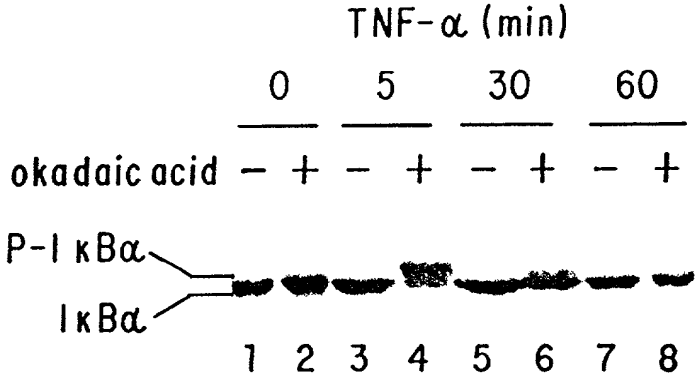
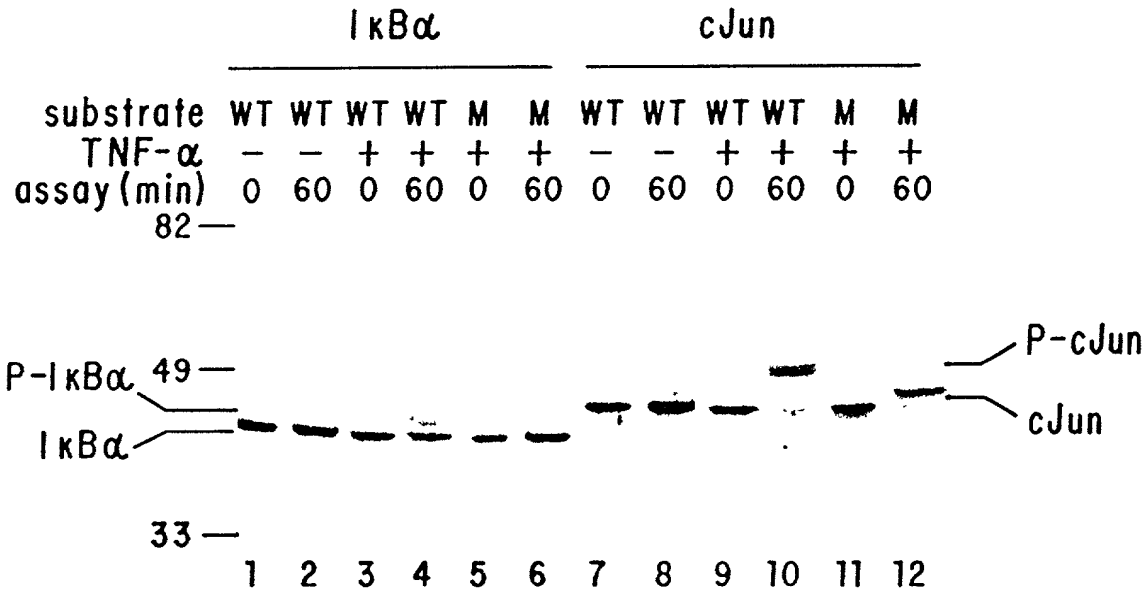


FIG.10B

FIG.10C



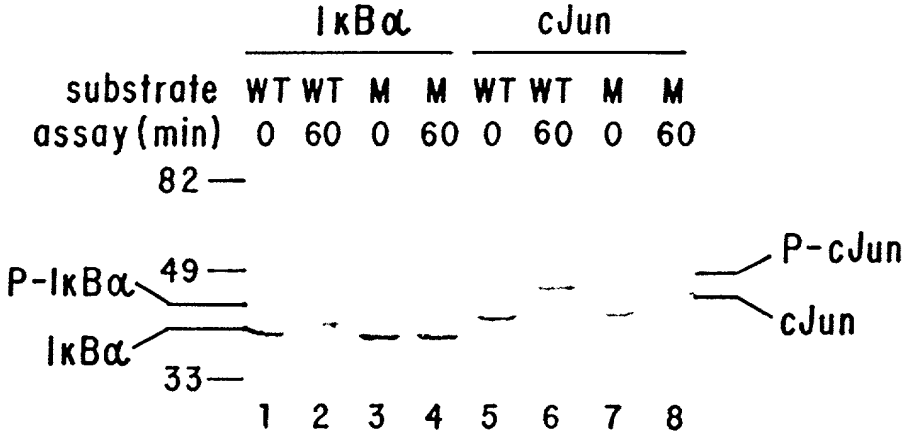


FIG.10D

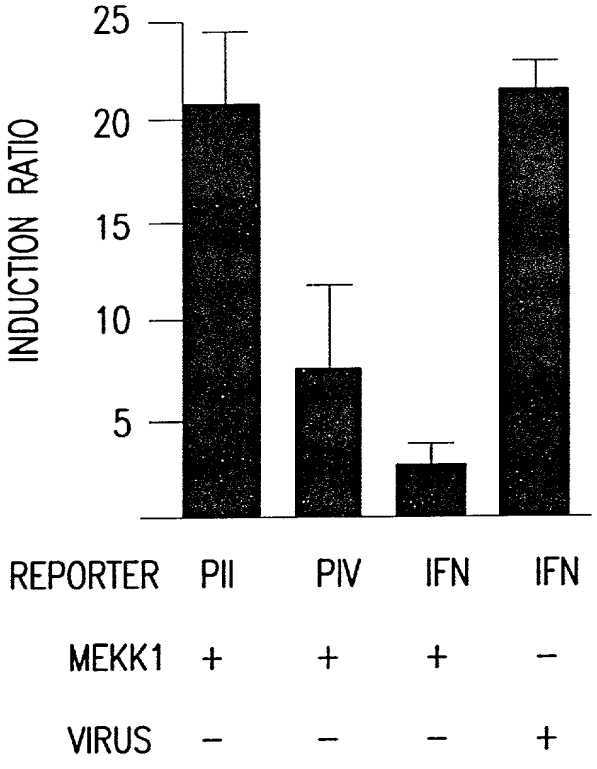


FIG. 11A

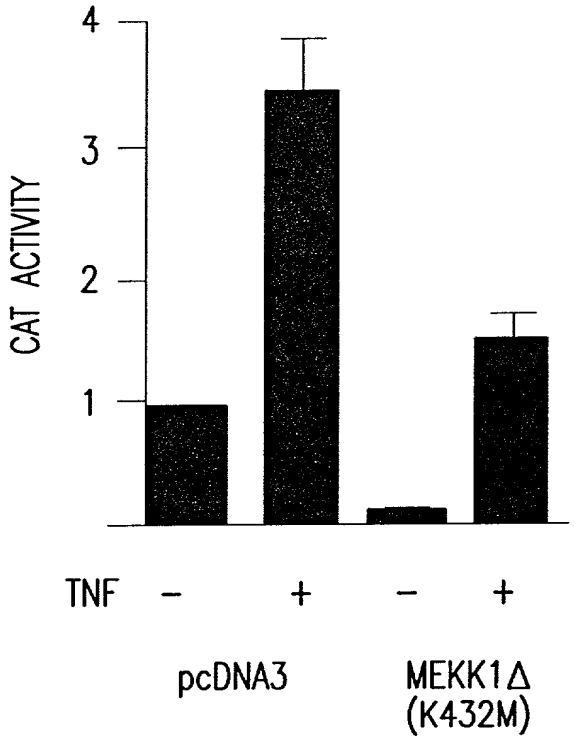


FIG. 11B

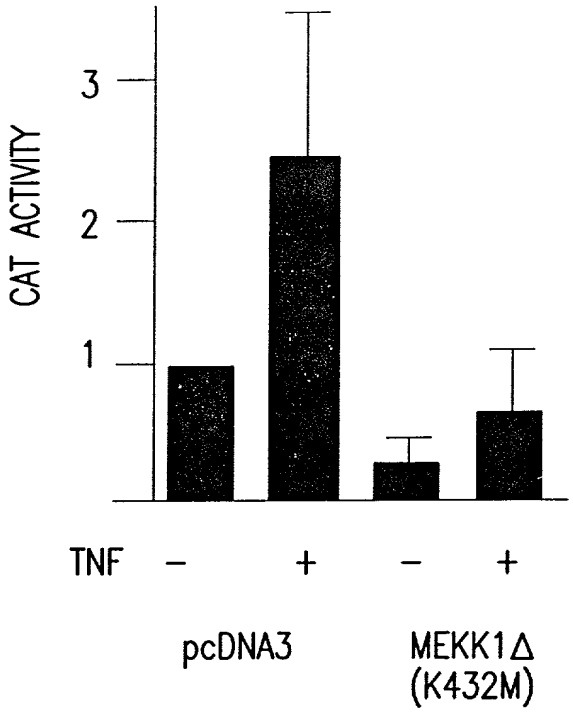


FIG. 11C

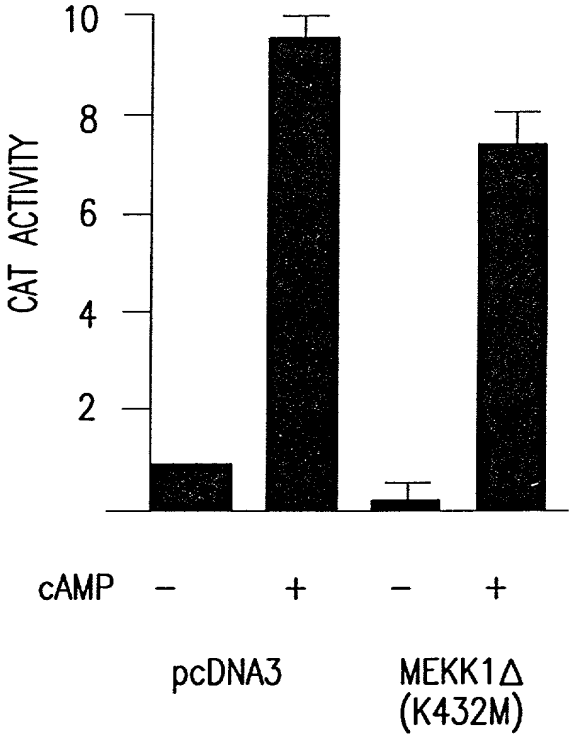


FIG. 11D

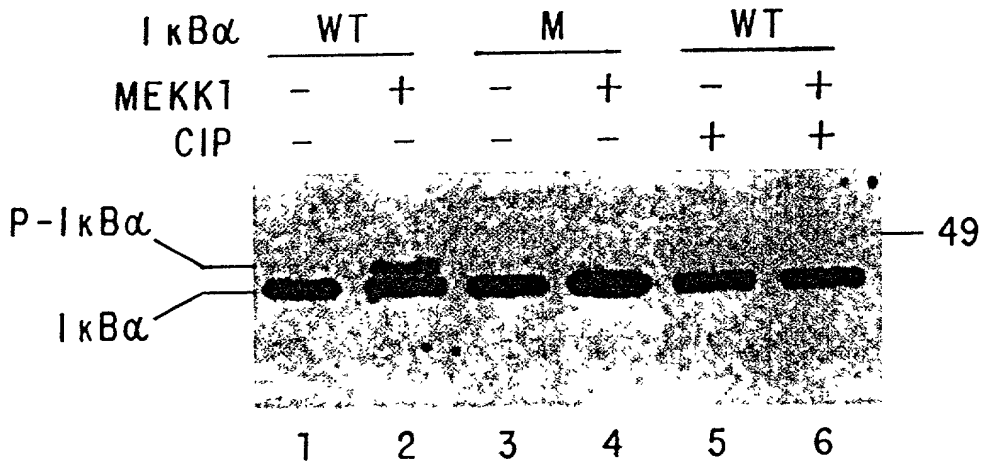


FIG.12

SUBSTITUTE SHEET

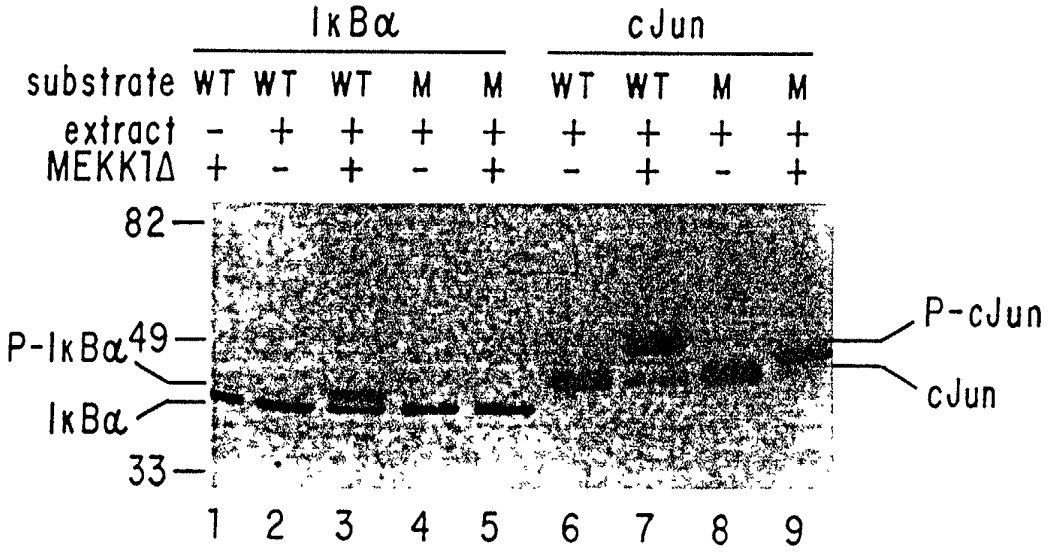


FIG.13A

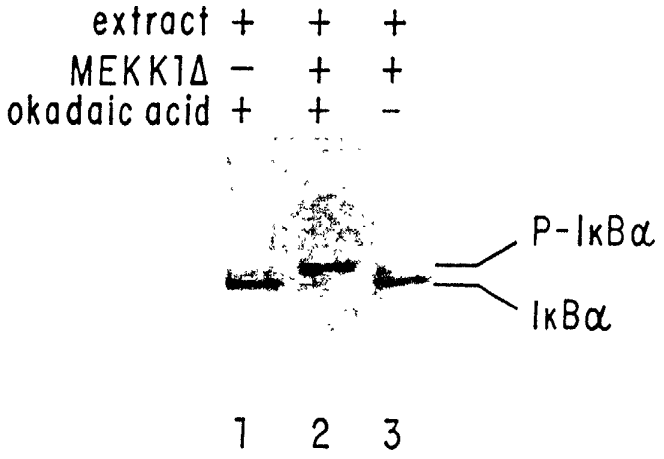


FIG.13B

20020703 SOGESOFT

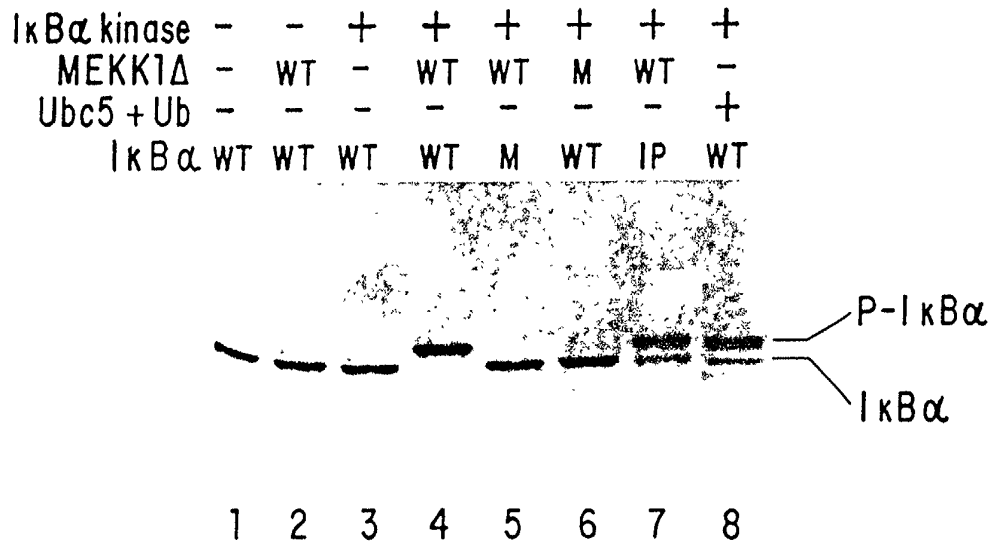


FIG.13C

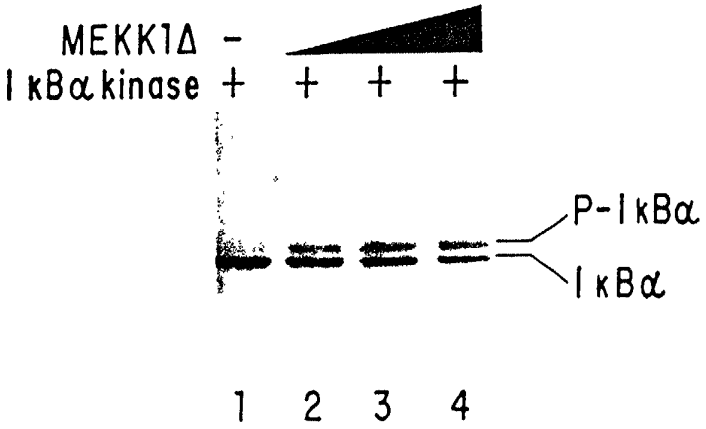


FIG.13D

FIG.14A

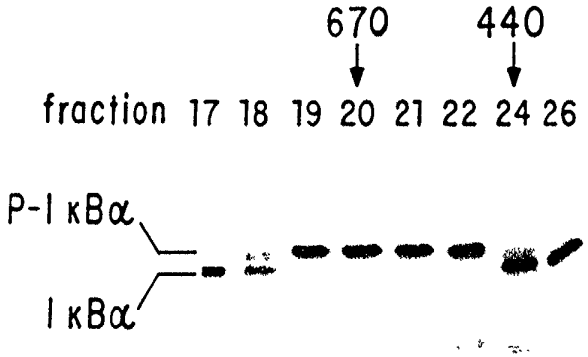
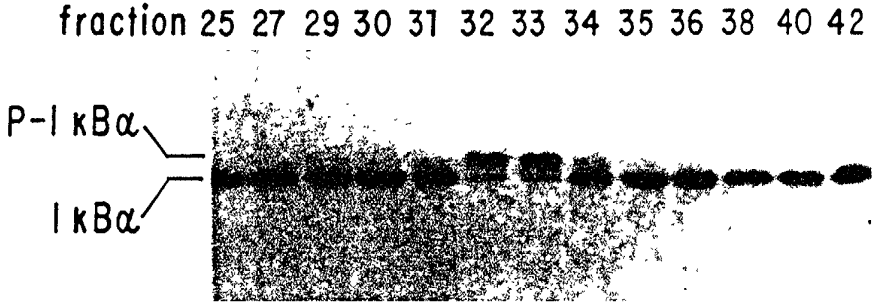


FIG.14B

FIG.14C



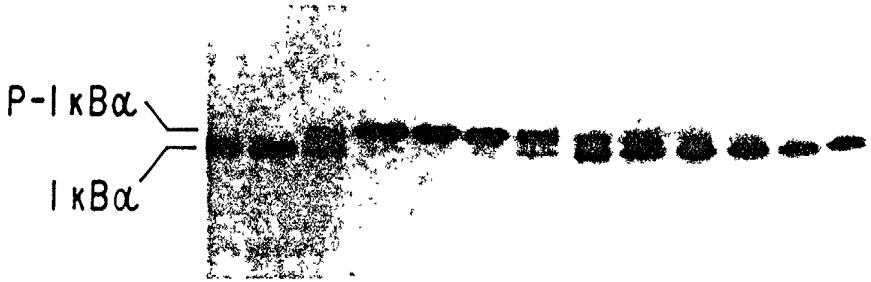


FIG.14D

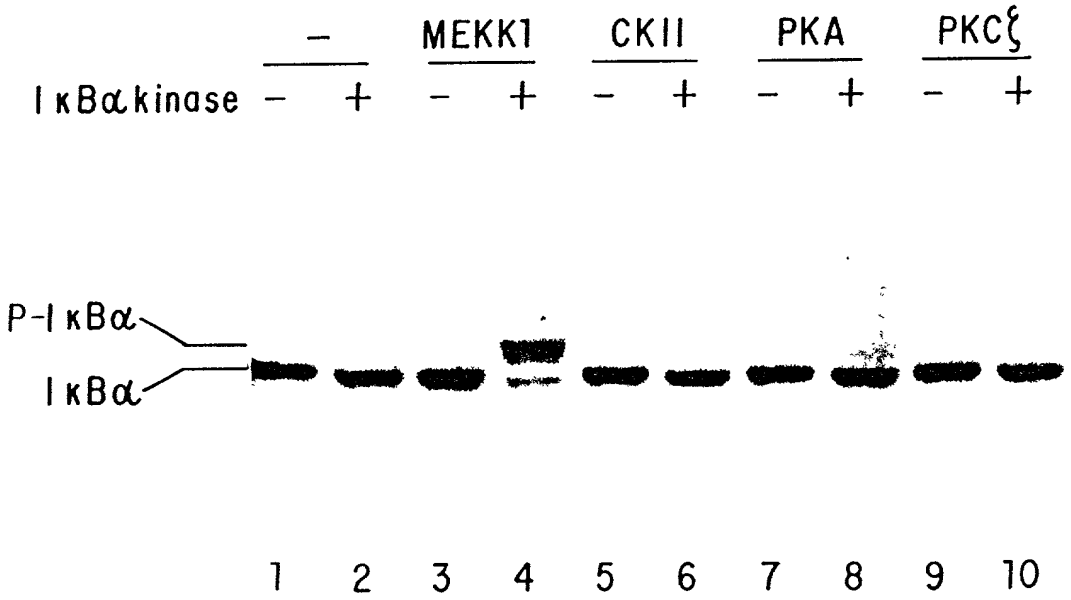


FIG.15A

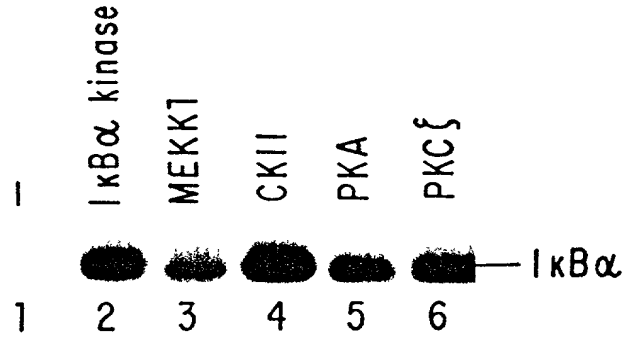


FIG.15B

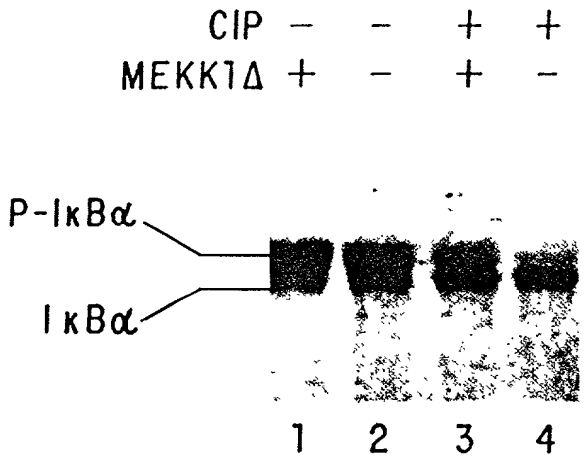


FIG.16A

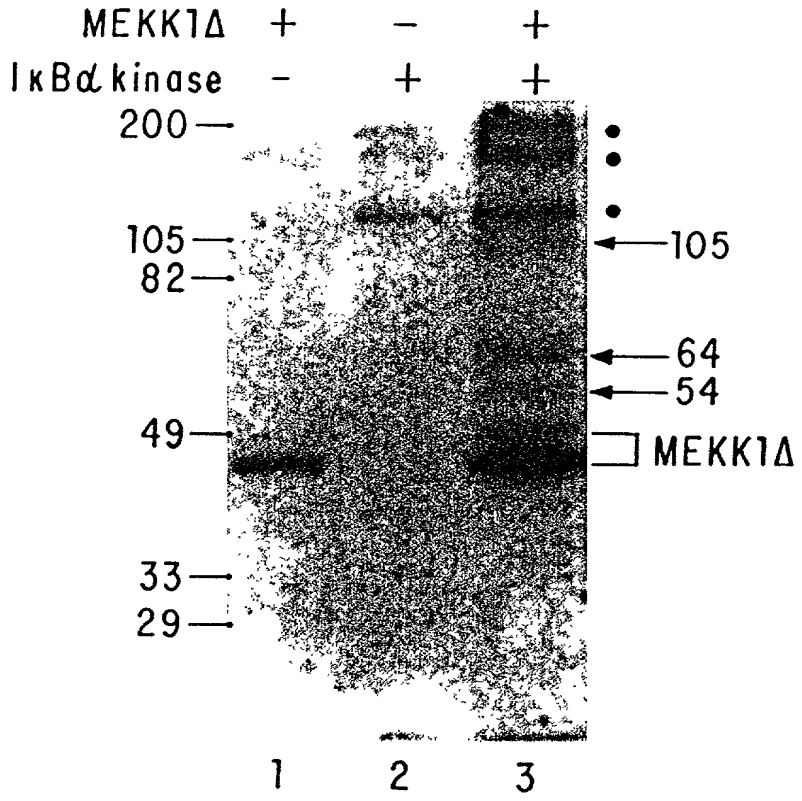


FIG.16B

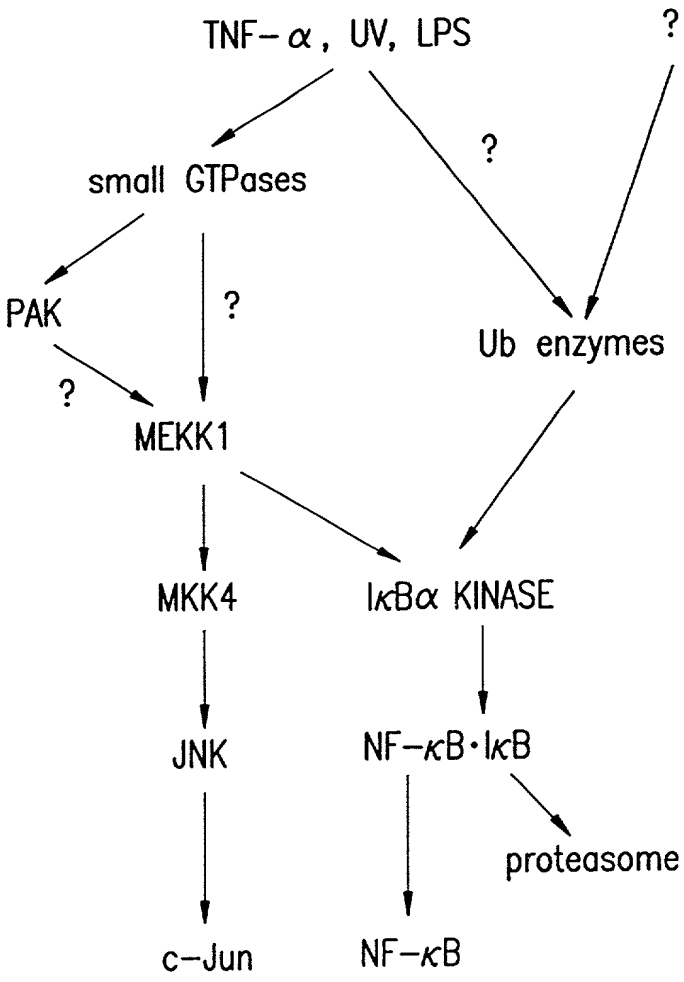

TNF-α, UV, LPS
?
small GTPases
?
PAK
?
Ub enzymes
?
MEKK1
MKK4
IκBα KINASE
JNK
NF-κB·IκB
proteasome
c-Jun
NF-κB

FIG. 17

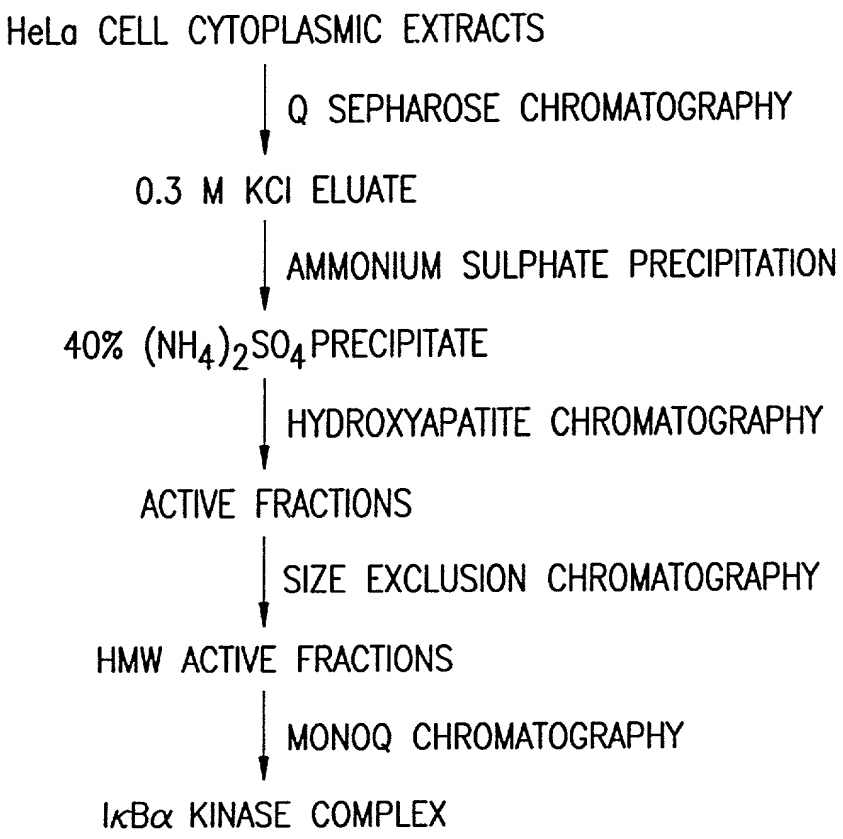

HeLa CELL CYTOPLASMIC EXTRACTS
Q SEPHAROSE CHROMATOGRAPHY
0.3 M KCl ELUATE
AMMONIUM SULPHATE PRECIPITATION
40% $(NH_4)_2SO_4$ PRECIPITATE
HYDROXYAPATITE CHROMATOGRAPHY
ACTIVE FRACTIONS
SIZE EXCLUSION CHROMATOGRAPHY
HMW ACTIVE FRACTIONS
MONOQ CHROMATOGRAPHY
IκBα KINASE COMPLEX

FIG. 18

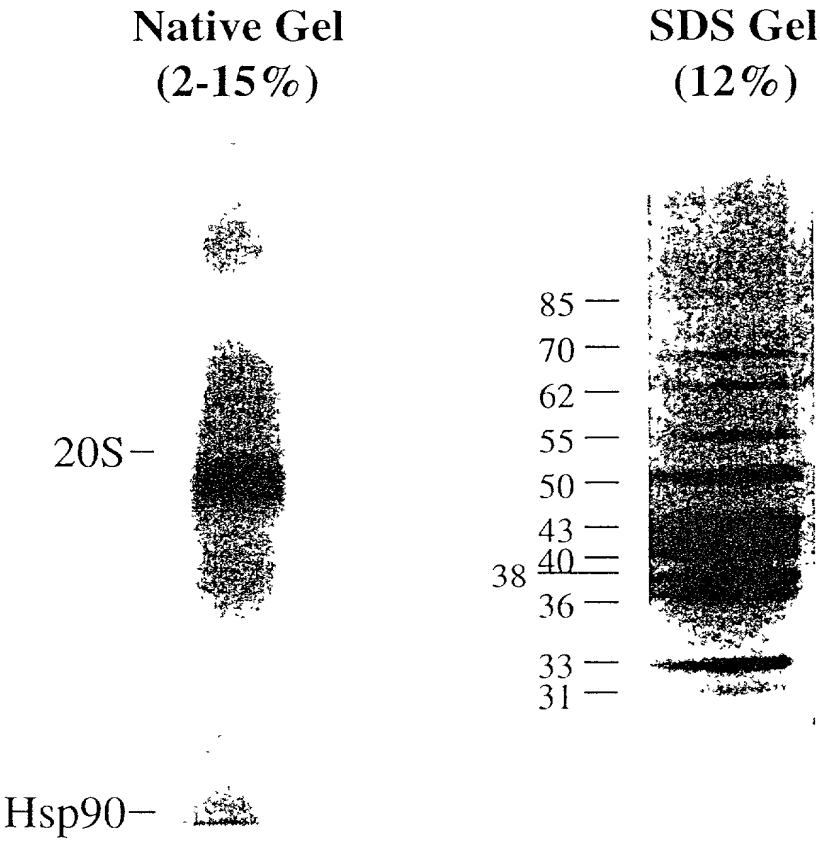


FIG.19A

FIG.19B

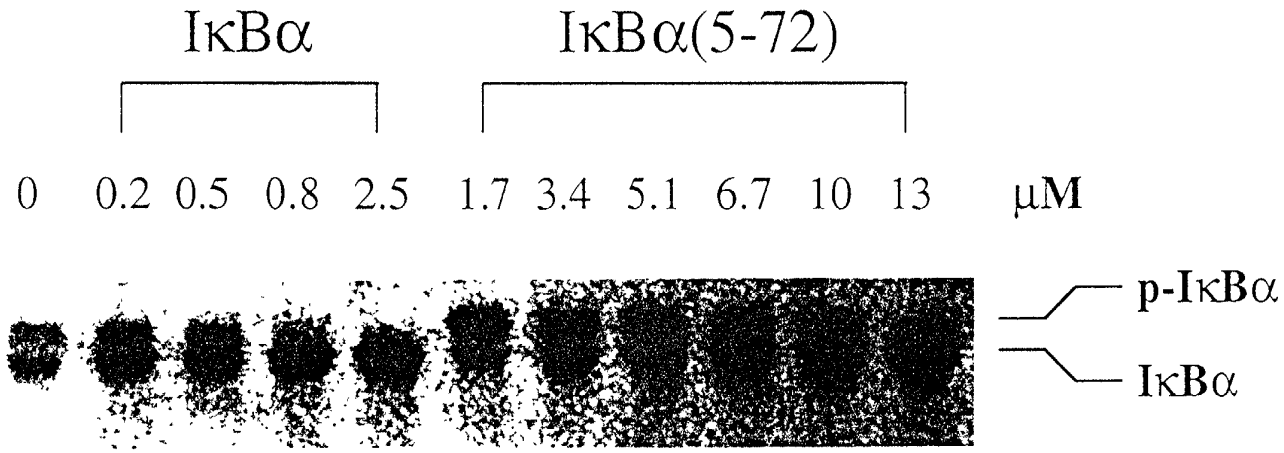


FIG.20

A
B
C
D

p50: pep1: [L/I] [Y] [V] [E] [L/I] [E] [R]
pep2: [L/I] [Q/K] [E] [V] [L/I] [E] [T] [L/I] [L/I] [S] [L/I] [E] [K*]
pep3: [L/I] [F] [T] [T] [M] [E] [L/I] [M] [R]

p40: pep4: [T] [Y] [H] [A] [L*] [S] [N] [L*] [P] [K*]

FIG.21

```
  1 gggtgacgag tggtggccga agcaggggga cagcaaggga cgctcaggcg gggaccatgg
 61 cggacggcgg ctcggagcgg gctgacgggc gcatcgtcaa gatggaggtg gactacagcg
121 ccacggtgga tcagcgccta cccgagtgtg cgaagtatgc caaggaagga agacttcaag
181 aagtcattga aacccttctc tctctggaaa agcagactcg tactgcttcc gatatggtat
241 cgacatcccg tatcttagtt gcagtagtga agntgtgcta tgaggctaaa gaatgggatt
301 tacttaatta aaaatattat tgctttttgt ccaaaaggcg gagtcaagtt aaaaacaagc
361 tagttgacaa aaaatggatt naacagttgc tgtnacttat tgttt
```

```
  1 ataccaagag gtaccaggaa gcattgcatt tgggttctca gctgctgcgg gagttgaaaa
 61 agatggacga caaagctctt ttggtggaag tacagctttt agaaagcaaa acataccatg
121 ccctgagcaa cctgccgaaa gcccgagctg ccttaacttc ttctcgaacc acagcaaatg
181 ccatctactg ccccctaaat tgcaggccac cttggacatg cagtcgggta ttatccatgc
241 agcagaagag aaggcttgaa actcgtactc atacttctat gaggcattta gggtatgact
301 catcgacagc ccaaggcatc aca
```

FIG.22

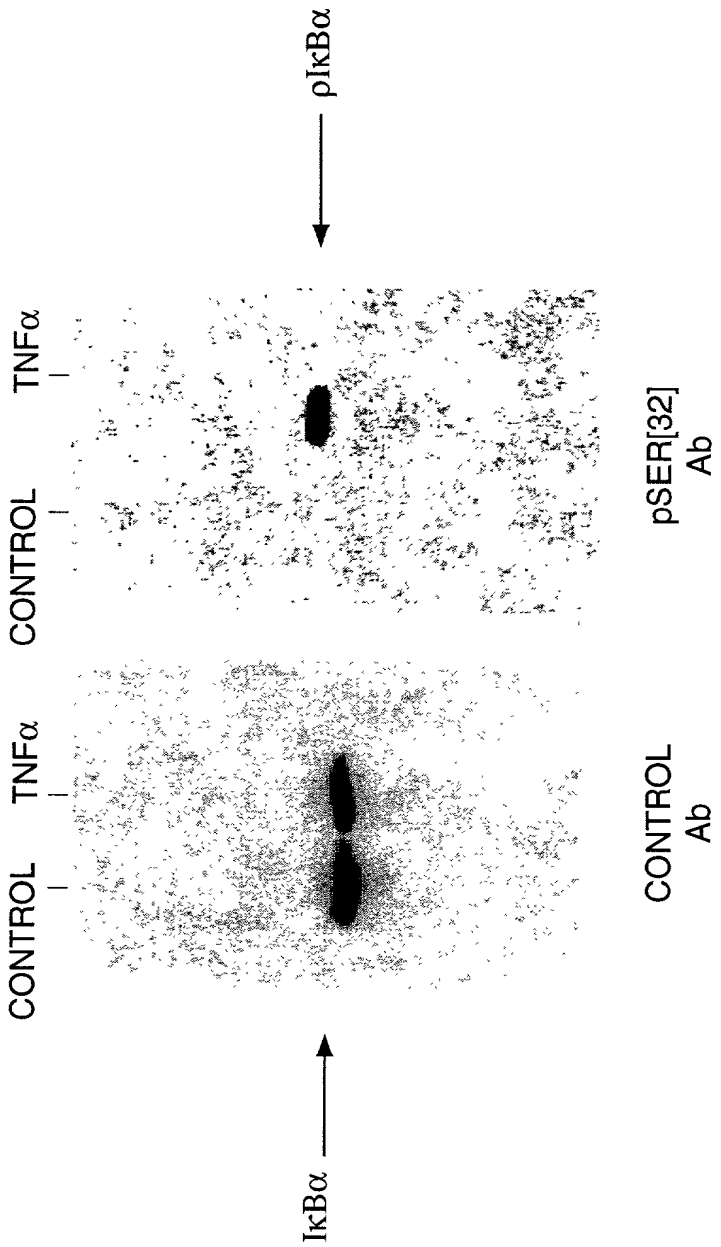


FIG. 23

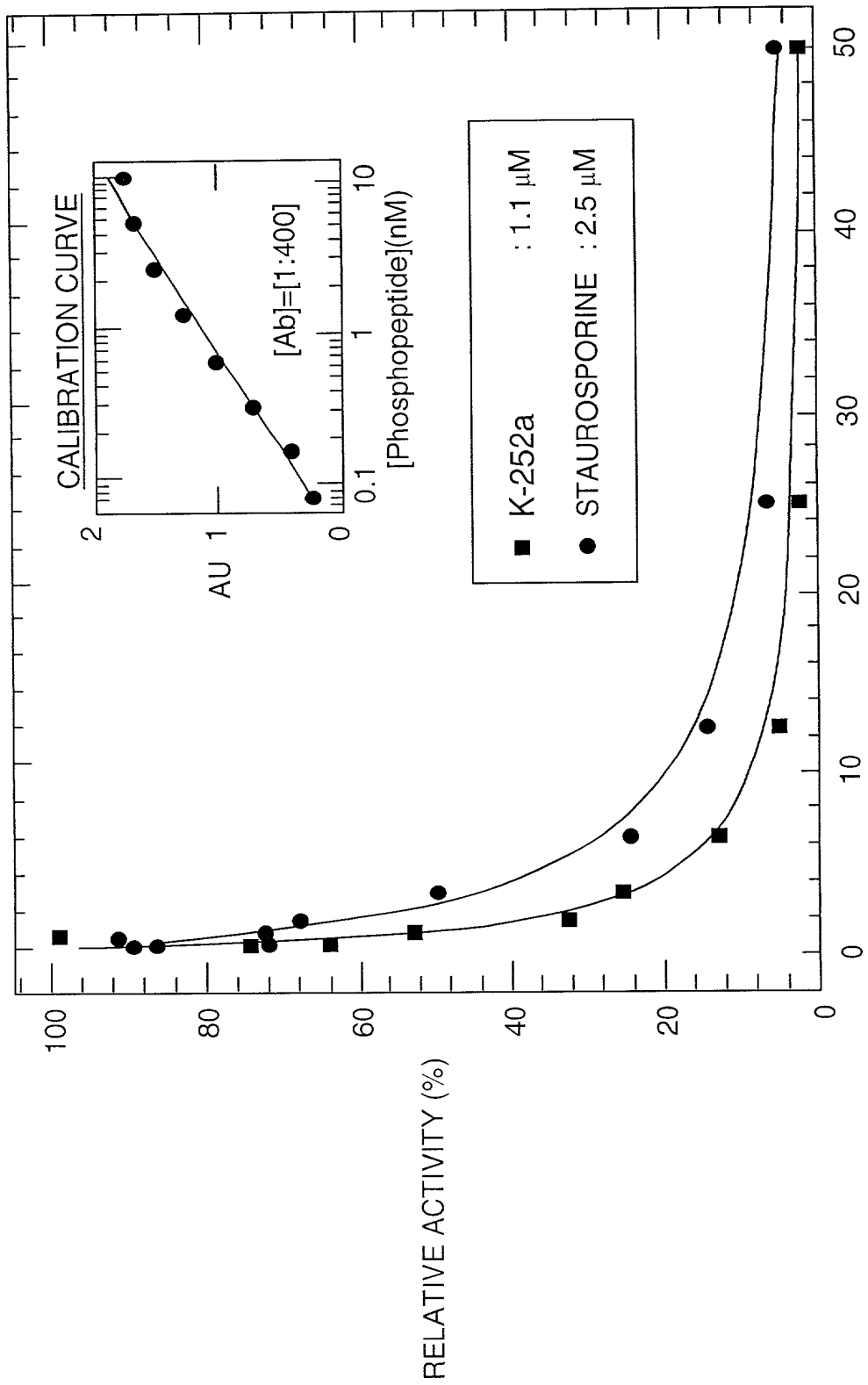

RELATIVE ACTIVITY (%)
100
80
60
40
20
0
0
10
20
30
40
50
[INHIBITOR] (μM)
K-252a : 1.1 μM
STAUROSPORINE : 2.5 μM
CALIBRATION CURVE
AU
2
1
0
0.1
1
10
[Ab]=[1:400]
[Phosphopeptide](nM)

FIG. 24